Seeing Is Believing: Extravascular Platelet Recruitment in Asthma and Allergic Inflammation

There is increasing recognition that platelets, in addition to their well-known role in hemostasis and thrombosis, contribute distinct functions in nonthrombotic processes such as innate immunity and inflammatory disorders (1, 2). Circulating activated platelets and platelet association with leukocytes (e.g., eosinophils) to form platelet–leukocyte complexes have been observed in asthma and other allergic and eosinophilic diseases, as well as in animal models of allergic inflammation (2–8). There is evidence that platelets are required for leukocyte recruitment in, for instance, asthma or allergic inflammation, by the association with leukocytes in the circulation and "licensing" transmigration of the leukocytes. There is also evidence that platelets may be associated with bronchospasm and airway remodeling (2–4, 6). In addition, platelet accumulation in response to an allergen has been reported in an animal model of allergic airway inflammation, where a proportion of tissue platelets were observed not to be associated with leukocytes (9). Similarly, platelet recruitment to the lung after LPS administration appears to occur independently of neutrophil recruitment (10). These latter studies indicate that recruitment of platelets themselves may be a process distinct from that of leukocytes. Consistent with this conclusion, platelets express chemokine receptors (11).

In this issue of the *Journal*, Shah and colleagues (pp. 557–568) describe their assessment of platelet recruitment to extravascular compartments in lungs of patients with asthma and in mice challenged with house dust mite in models of allergic inflammation, and they detail the roles of chemokines and their receptors in the recruitment process (12). They detected increased platelets scattered throughout the epithelium and submucosa of bronchial biopsies from patients with asthma compared with biopsies from subjects without asthma. Platelets were observed in larger numbers in lung parenchyma in autopsies from patients with fatal asthma. In sensitized mice, allergen exposure induced recruitment of eosinophils and platelets into the airway, and induced platelet rolling and adhesion on endothelium in cremaster muscle vessels visualized by intravital microscopy, accompanied by an increase in extravascular platelets. Human platelets migrated toward CCL11 (eotaxin-1) *in vitro*. Supporting a role for CCL11, airway smooth muscle from patients with moderate asthma was shown to release more CCL11 compared with smooth muscle from normal subjects, consistent with the literature (13). Furthermore, studies on effects of selective chemokine receptor antagonists demonstrated that platelet migration in the cremaster vessel model was dependent on the CCL11 receptor CCR3. Migration was not associated with platelet aggregation or complex formation with leukocytes.

Strengths and novel aspects of the article by Shah and coworkers include the analysis of bronchial biopsies from subjects with asthma of different severities, analysis of tissue samples from fatal asthma (most of whom had allergic asthma), and the combination of patient and mouse experiments. One limitation relates to the relevance of the cremaster muscle allergic inflammation model to asthma. Despite being an elegant and advantageous method for intravenous preadministration of the anti-CD49b antibody, intravital microscopy, and real-time video capture, the relevance of platelet rolling, adhesion, and accumulation in cremaster muscle to the lung vasculature and airway inflammation in asthma is uncertain. Another potential limitation is quantification of platelets in human and murine tissues by immunochemical localization of CD49b (ITGA2). A large literature suggests that CD49b, the α subunit of the ITGA2/ITGB1 complex, which is the platelet collagen receptor and highly expressed on platelets, is not platelet specific, as some tissue cells (e.g., fibroblasts) express CD49b (14). However, it appears that no significant "background" staining of other cells was detected, and the size and morphology of platelets also allowed them to be identified and distinguished from other elements.

The results indicate that platelet recruitment is not associated with the classical platelet aggregation of hemostasis and thrombosis and that it also may occur independently of leukocyte adhesion and recruitment. The dynamics of platelet migration remain incompletely understood and it may thus be a different process from the well-documented intracellular platelet–leukocyte complex formation during inflammation and in patients with asthma associated with the extravasation of leukocytes, primarily eosinophils (*see* above). However, based on the study by Shah and others, we do not yet know whether the intravascular association of platelets with leukocytes also leads to the extravasation of "whole" heterogeneous (platelet–leukocyte) complexes and if platelets later dissociate from such complexes after transmigration in addition to extravasating as single entities.

It would be interesting in future studies of patient biopsies at different times after allergen exposure to examine the dynamics and extent of platelet migration and to associate migration with features of asthma and the response to challenge. Collection and analysis of tissue samples from a greater number of patients with asthma of different severity and potentially also of different disease phenotypes (e.g., type 2 immunity–high or –low asthma) may also further elucidate these phenomena.

In conclusion, Shah and colleagues have made novel observations of single, extravascular platelets in the lung tissue of patients with asthma as well as in the lungs of mice after allergen challenge. Extravascular platelet recruitment, at least in a mouse allergic inflammation model, appears to be CCR3 dependent,

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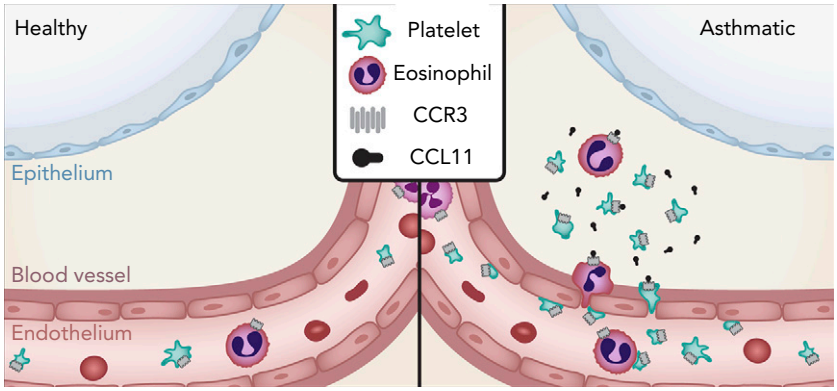

Figure 1. Proposed scenario with platelets and leukocytes (e.g., eosinophils) in asthmatic lung (to the right) compared with nonasthmatic lung (to the left). Previous studies have indicated that platelets associate with leukocytes such as eosinophils to form complexes in the circulation in asthma and other allergic or eosinophilic diseases, leading to leukocyte, primarily eosinophil, extravasation at sites where the endothelium is locally activated by type 2 immunity mediators. In addition, as now described by Shah and colleagues, single platelets are also recruited and appear extravascularly in the lung of patients with asthma or in an allergic airway inflammation model after challenge. Shah and others suggest, partly based on a mouse cremaster model of allergic inflammation, that such platelets roll, adhere, and are recruited in a CCR-dependent manner, independently of interaction with leukocytes.

implicating CCL11, other eotaxins, or other CCR3 ligands as likely or possible platelet chemoattractants *in vivo* (Figure 1). Thus, even if platelet recruitment is independent of leukocyte recruitment, platelets may use similar chemokines and receptors as do eosinophils in asthma (13). The results support the involvement of platelets in asthma and allergic disease in addition to or unrelated to their role in hemostasis and thrombosis. Further understanding of the mechanisms of platelet migration and the roles of platelets in allergic inflammation distinct from those in hemostasis may yield new therapeutic targets (15, 16). ■

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